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(54) Title: TARGETED VIRUS			

(57) Abstract

Genetically engineered viruses which comprise a genetically engineered gene and having a chimeric envelope protein which specifically binds to a target cell receptor, to which the naturally occurring envelope protein does not bind, may be used for cell type-specific gene therapy. Transduction of human stem and/or progenitor cells with such viruses containing chimeric envelope proteins which specifically bind to human stem and/or progenitor cells proceeds with high specificity and efficiency, especially when such viruses are incubated with human stem and/or progenitor cells cultured under conditions of rapid medium exchange.

Description

Targeted Virus

Technical Field

The present invention relates to a virus which has an chimeric envelope protein on its surface that effects the specific binding of the virus to a cell of interest. The present invention also relates to a method of producing such a virus and methods of transducing cells and gene therapy utilizing such a virus. The present invention further relates to stem and/or progenitor cells which have been transduced with such a virus and a packaging cell line which produces such a virus.

Background Art

Genetically transformed human stem cells have wide potential application in clinical medicine, as agents of gene therapy. Gene therapy is an emerging approach to clinical treatment which has evolved from earlier approaches in medical care. The earliest approaches to medical care, evolving over centuries, include gross surgical procedures and the administration of crude mixtures as medicinal agents. In the past century, biochemical pharmacology has supervened as the major method of medical treatment. Under this paradigm, pure pharmaceutical molecules are delivered to the patient. In general, such pharmacologic agents act either as poisons (such as antimicrobials or cancer chemotherapy agents), physiologic mimetics which stimulate endogenous receptors (e.g., opiates, adrenergic agonists), or physiologic antagonists which block endogenous receptors (e.g. antihypertensives, anesthetics).

The principle underlying gene therapy is to, rather than deliver doses of pharmacologic molecules, deliver a functional gene whose RNA or protein-product will produce the desired

biochemical effect in the target cell or tissue. There are several potential advantages of gene therapy over classical biochemical pharmacology. First, inserted genes can produce extremely complex molecules, including DNA, RNA and proteins, which can be extraordinarily difficult to administer and deliver themselves. Next, controlled insertion of the desired gene into specific target cells can control the production of gene product to defined tissues. Finally, gene therapy can in principle be permanent within an individual, as the gene will continue to function in the target cells and their progeny.

However, there are several problems that must be addressed for successful gene therapy. The first is to be able to insert the desired therapeutic gene into the chosen cells. Second, the gene must be adequately expressed in the target cell, resulting in the appropriate levels of gene product. Finally the DNA, RNA or protein produced must be properly processed by the target cell so that it is functional, i.e. so that gene therapy actually confers clinical therapy. Several methods of gene insertion into human cells *in vitro* have been accomplished and are listed in Table 1.

Table 1: Comparison of DNA transfer methods.

Variable	Microinjection	Electroporation	Retrovirus
Efficiency	10-100%	0.0001-1%	1-100% (depends on titer)
Effort	High	Low	Intermediate
Expense	High	Low	Intermediate
Stability	Good	Good	May be inactivated or become infective
DNA synthesis	?	?	Required
Size of DNA input	Not restricted	Not restricted	Limited (<6 to 8 kb)
Need Extraneous DNA	No	No	Yes

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Other techniques, such as homologous recombination, are being developed as well in many laboratories. With these issues in mind, research on gene therapy has been on-going for several years. This research, which began in several types of cells in vitro, has progressed to animal studies, and has recently entered the first human clinical trials (Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF et al., Proc. Natl. Acad. Sci. U.S.A., 1990; 87:473-7; Culver K, Cornetta K, Morgan R, Morecki S, Aebersold P, Kasid A, Lotze M, Rosenberg SA, Anderson WF, Blaese RM, Proc. Natl. Acad. Sci. U.S.A., 1991; 88:3155-9.) Tissue-specific expression of a functionally rearranged $\lambda 1$ Ig gene by transduction with a retrovirus vector has been observed (Cone RD, Reilly EB, Eisen HN, and Mulligan RC, Science, 236:954-957 (1987)).

The hematopoietic system is an ideal choice as a delivery system for gene therapy. Hematopoietic cells are readily accessible, simply by bone marrow aspiration or by peripheral blood mononuclear cell harvest. Once the genetic insertion is accomplished in vitro, the treated cells can be reinfused intravenously, after which the genetically transformed cells will home to and develop in the bone marrow. Since mature blood cells circulate throughout the body, the genetically modified cells would deliver the specific gene product to any desired tissue. Most importantly, hematopoietic tissues contain stem cells, which possess extensive (perhaps unlimited) capacities for self-renewal. This implies that if genetic material were stably transduced into these stem cells, then upon reinfusion of the hematopoietic tissue, these altered stem cells can expand and repopulate the marrow with cells that express the new gene. This would lead to longlasting, perhaps lifelong delivery of the desired gene product. Similarly, successful stable gene transfer into stem cells located in other tissues, or into embryonic stem cells,

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would likewise lead to longlasting gene product delivery.

Successful hematopoietic stem cell gene therapy has broad application, to both diseases specific to the hematopoietic system and to other organ system diseases. Within the hematopoietic system, both inherited and acquired diseases can be treated by stem cell gene therapy. For example, hemoglobin deficiencies such as α and β Thalessemias could be treated by the insertion of the gene coding for the globin α or β chain, together with regulatory sequences that confer high level tissue-specific expression of these genes in erythrocytes (Grosveld F, van Assendelft GB, Greaves DR, Kollias G, Cell, 51:975-985;1987; Soriano P, Cone RD, Mulligan c, Jaenisch R, Science, 234: 1409-1413; 1986). Similarly, sickle cell anemia could be corrected by the genetic insertion of the fetal globin gene into hematopoietic stem cells, as the regulated expression of high levels of fetal hemoglobin are sufficient to prevent sickling in red cells despite the copresence of sickle hemoglobin (Sunshine HR, Hofrichter J, et al., J. Molec. Biol. 133:435; 1979). Genetic diseases of neutrophils caused by functional protein deficiencies, such as leukocyte adhesion deficiency (LAD) or chronic granulomatous disease (CGD) could be treated by the genetic insertion of the gene encoding the defective or absent gene, along with regulatory DNA sequences that confer high level, tissue specific expression into hematopoietic stem cells (Wilson JM, Ping AJ, Krauss JC, Mayo-Bond L, Rogers CE, Anderson DC, Todd RF, Science, 248:1413-1416; 1990). Genetic diseases involving platelets, such as von Willebrand's Disease, could be corrected by the genetic insertion of the gene encoding, e.g. von Willebrand's Factor, along with sequences which permit its expression and secretion.

The particular suitability of hematopoietic stem cell gene therapy for the replacement of congenitally deficient

gene products is particularly evident in the treatment of lymphocyte immunodeficiency diseases, such as severe combined immunodeficiency (SCID) due to adenosine deaminase deficiency. Retroviral gene therapy of circulating T cells with the ADA gene has been found to be successful at reducing the clinical immunodeficiency experienced by these patients, but the effects are only temporary, because the transfected T lymphocytes have a finite life span in vivo (Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF, et al., Proc. Natl. Acad. Sci. U.S.A., 1990;87:473-7; Culver K, Cornetta K, Morgan R, Morecki S, Aebersold P, Kasid A, Lotze M, Rosenberg SA, Anderson WF, Blaese RM, Proc. Natl. Acad. Sci. U.S.A., 1991; 88:3155-9). If, however, the gene could be successfully transfected into hematopoietic stem cells, then all of the T cells which arose from these stem cells would contain and express the ADA gene. Therefore, since the transfected stem cells would persist and proliferate for the life of the patient, the T cell ADA deficiency would be permanently treated by a single gene transfer stem cell treatment (Wilson JM, Danos O, Grossman M, Raulet DH, Mulligan RC, Proc. Natl. Acad. Sci. U.S.A., 87:439-443;1990).

In addition to treating inherited enzymatic abnormalities of the hematopoietic system, stem cell gene therapy could be useful for protecting stem cells and their progeny from toxic exogenous agents such as viruses or chemotherapy. For example, gene transfer of DNA sequences encoding the TAR binding site of the HIV TAT transactivating factor have been shown to protect T cells from spreading infection by the HIV virus (Sullenger BA, Gallardo HF, Ungers GE, Gilboa E, Cell, 1990;63:601-8). Stable transfection of these sequences into hematopoietic stem cells would result in a pool of T cells, all arising from these stem cells, which would be relatively or absolutely resistant to the spread of HIV.

Similarly, successful transfection of the genes encoding the multi-drug resistance gene (MDR) or the methotrexate resistance gene into human bone marrow stem cells would create stem cells which would be relatively resistant to the affects of cancer chemotherapy. Following autologous bone marrow transplantation with these genetically manipulated cells, patients would be able to tolerate chemotherapy with the agents to which their stem cells were protected without suffering the profound bone marrow suppression commonly caused by these anti-cancer drugs. This would enable patients to receive more effective doses of cancer chemotherapy with less toxicity.

One can readily envision that hematopoietic stem cell gene therapy will also be useful for acquired hematopoietic diseases such as leukemia, lymphoma and aplastic anemia. Once the genetic causes of these diseases are discovered, insertion of a gene whose product either overcomes that of the abnormal gene in the cell or corrects it directly (e.g., by splicing out and replacing the gene, via homologous recombination) would correct the abnormality.

On a broader level, however, hematopoietic stem cell gene therapy would be useful for the treatment of diseases outside the hematopoietic system as well. Gene transfer of DNA sequences carrying therapeutic soluble proteins could give rise to mature blood cells which permanently secrete the desired amounts of a therapeutic molecule. By way of examples, this approach could be useful for the treatment of, e.g., diabetes mellitus by the insertion of DNA sequences for insulin along with regulatory DNA sequences that control the proper expression of the transfected insulin gene, perhaps in response to elevated plasma glucose levels. Systemic hypertension could be treated by genetic insertion of stem cells with DNA sequences encoding secretory peptides which act

as competitive inhibitors to angiotensin converting enzyme, to vascular smooth muscle calcium channels, or to adrenergic receptors. Alzheimer's disease could possibly be treated by genetic insertion into stem cells of DNA sequences encoding enzymes which break down amyloid plaques within the central nervous system.

The many applications of gene therapy, particularly via stem cell genetic insertion, are thus well known and have been extensively reviewed (Boggs SS, Int. J. Cell Cloning, 8:80-96; 1990; Kohn DB, Anderson WF, Blaese RM, Cancer Investigation, 7:179-192; 1989; Lehn PM, Bone Marrow Transplantation, 5:287-293; 1990, Verma IM, Scientif Amer., pp. 68-84; 1990; Weatherall DJ, Nature, 349:275-276; 1991; Felgner PL, Rhodes G, Nature, 349:351-352; 1991). There are indeed increasing examples of success in achieving therapeutic gene transfer into differentiated human cells, as described, for example, in T lymphocytes (Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF, et al., Proc. Natl. Acad. Sci. U.S.A., 1990;87:473-7; Culver K, Cornotia K, Morgan R, Morecki S, Aebersold P, Kasid A, Lotze M, Rosenberg SA, Anderson WF, Blaese RM, Proc. Natl. Acad. Sci. U.S.A., 1991; 88:3155-9).

Unfortunately, achieving gene transfer into human stem cells has not until now been accomplished. While several groups have demonstrated the feasibility of retroviral mediated gene transfer into human hematopoietic cells, human primitive hematopoietic stem cells have not been successfully transfected. This is in sharp contrast to experiments in the mouse, in which some level of retrovirally mediated gene transfer into hematopoietic stem cells has been possible (Wilson JM, Danos O, Grossman M, Raulet DH, Mulligan RC, Proc. Natl. Acad. Sci., U.S.A., 87:439-443; 1990).

The major impediments to achieving successful human hematopoietic stem cell gene therapy has been the inability to have cultured stem cells replicate to allow for the stable insertion of genes, and the existence of a retroviral packaging cell line which efficiently infects human stem cells. Successful infection with a retroviral vector requires: binding of the virus to the target cell, penetration into the cell, uncoating of the virus, reverse transcription of viral RNA into DNA, integration of the viral DNA into the host chromosome (using a viral integrase), and expression of the gene of interest. Successful stable gene insertion via retroviral vectors into a target cell requires that the target cell undergo at least one round of cell division (Springett GM, Moen RC, Anderson S, Blaese RM, Anderson WF, J. Virol., 1989;63:3865-9). Thus, if stem cells are not dividing in the presence of the desired genetic material, the material will not be stably inserted into the stem cells. Part of the difficulty in infecting stem cells is the retroviral delivery system itself.

A retrovirus is an RNA virus consisting of approximately 9,000 bases of RNA. After infection of the host cell, the RNA is converted into double stranded DNA which inserts into the host chromosome. The integrated viral DNA is called the provirus. The provirus consists of two repetitive sequences, called the long terminal repeat (LTR), at the beginning (5') and the end (3') of the virus sequences. The 5' LTR directs transcription of virus mRNA's which encode at least two transcripts which encode the viral structural and replication proteins. The first mRNA encodes the GAG and POL/INT proteins, and the second mRNA encodes the envelope proteins. Some viruses also encode transcripts involved in other virus replication functions, for example the HIV REV gene.

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The host and cell type specificity of the retrovirus is determined in some cases by the ability of the LTR to function in the specific cell and in other cases by the presence of the receptor for the viral envelope on the cell. In the latter case, the cell receptor binds to the viral envelope (ligand), and the virus enters the cell. It is felt that lentiviruses, such as the HIV retrovirus, enter the cell via virus envelope mediated fusion after the viral envelope binds to the CD4 molecule on the cell surface (Grewe C, Beck A, and Gelderblom HR, J. AIDS, 3, 965-979, 1990). On the other hand, the amphotropic murine retroviruses (MoMLV), which are used in current packaging cell lines, may enter the host via a pH-independent envelope fusion with endosomal particles (McClure MO, Sommerfelt MA, Marsh M, and Weiss RA, J. Gen. Virol., 71, 767-773, 1990). The amphotropic virus envelope binds to a cell surface receptor present on all cells.

The virus particle is assembled at the cell membrane. The structural proteins, the GAG proteins in the case of MoMLV, and replication proteins (RT/INT) bind to cis-acting sequences present in viral RNA, and assemble at the cell surface with the virus envelope proteins and the virus particle buds off the cell surface.

It has been shown that the envelope proteins from a different virus will assemble virus particles in conjunction with the GAG and POL proteins of a second virus (Page KA, Landau NR, and Littman DR, J. Virol., 64, 5270-5276, 1990).

In order to accomplish gene therapy, the packaging virus must be able to efficiently infect the host cell. The majority of packaging lines developed to date are based on the mouse MoMLV amphotropic virus. Although this viral coat will bind to human cells, it is very inefficient at infecting human hematopoietic cells (Gruber DE, Finley KD, Hershberg RM,

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Katzman SS, Laikind PK, Siegmiller JE, Friedmann T, Vee JK and Jolley DJ, Science, 230, 1057-1061, 1985; Hogge DE & Humphries RK, Blood, 69, 611-617, 1987). Attempts to use a xenotropic virus (a virus which infects species other than mouse) packaging cell line have been hindered by very low viral titers of 3T3 xenotropic packaging cell lines. Finally, a Gibbon Ape Leukemia Virus envelope, which recognizes a widely distributed cell receptor, has been used to make a 3T3 pseudotype packaging cell line (Miller D et al., J. Virol., 65, 2220-4, 1991).

The ideal packaging virus would have an envelope (ligand) which recognizes a receptor present only on the subset of cells which are the intended target for gene therapy. This cell type specificity would have multiple advantages over the present amphotropic and xenotropic packaging cell lines which recognize a receptor present on all cell types. For example, human hematopoietic stem cells probably only represent .01% or less of mononuclear bone marrow cells. In addition, these cells appear to grow only when in contact with stromal cells, which represent a large proportion of bone marrow cultures. Thus, when attempting gene transfer in in vitro hematopoietic cultures, extremely high viral titers are required to infect a stem cell. These high viral titers are very difficult to obtain. Alternatively, enrichment of the mononuclear bone marrow for stem cells is possible, but the best method is by FACS which is very expensive and time consuming. In addition, the FACS sorted cells probably also require culture on a stromal layer which will also bind virus particles and effectively lower the virus titer.

Additionally, the ideal packaging line would be constructed using a human retrovirus envelope, since these viruses have evolved to efficiently infect human cells. Unfortunately, the known human retroviruses (HTLV and HIV)

only infect T-cells. In the case of HIV, T-cell specificity is due to the fact that only T-cells express the viral receptor on their cell membranes. However, studies have shown that the HIV envelope will form virions with the GAG and POL proteins of an amphotropic virus (Wilson K, Reitz MS, Okayama H, and Eiden MB, J. Virol., 63, 2374-8, 1989).

U.S. patent no. 4,731,324 describes a viral lysis assay in which modified viral particles are utilized. Specifically, the virus particles contain on their surface non-viral anti-analyte molecules which are composed of an anti-analyte which is derivatized with a lipid moiety which anchors the molecule to the virus lipid bilayer surface.

U.S. patent no. 4,948,590 discloses avidin- or streptavidin-conjugated liposomes which can be utilized as a vehicle for site-specific targeting of encapsulated drugs or other macromolecules, including retrovirus vectors. The conjugated liposomes are prepared by coupling, e.g., the carboxyl residues of streptavidin to the phospholipid amino groups of the liposome.

U.S. patent no. 4,786,590 describes the use of monoclonal antibodies which bind to one or more determinant sites of cell surface membrane receptors (CSMR) for directing agents to the site of transformed cells. Disclosed examples of such agents include radionuclides, toxins, and factors involved in complement lysis.

U.S. patent no. 4,701,416 teaches the use of protein carriers to which is conjugated an amino acid sequence which is homologous to at least a portion of gp70 envelope protein of FeLV as a FeLV immunogen. Disclosed examples of such carrier proteins include keyhole limpet hemocyanin, ovalbumin, porcine thyroglobulin, and bovine serum albumin.

U.S. patent no. 4,861,588 describes hepatitis B vaccines containing an amino acid chain corresponding to a chain of amino acids in the pre-S region linked to a carrier. Lipid vesicles are described as the preferred carrier.

U.S. patent no. 4,859,769 teaches the use of liposomes which may be tagged with a viral component which recognizes the second-step receptor on the surface of a cell. The liposome may be used to carry a toxic drug.

U.S. patent no. 4,545,985 discloses a method for modifying *Pseudomonas* exotoxin for conjugating the exotoxin to a monoclonal antibody which binds to a cell surface membrane receptor to form an immunotoxin.

However, none of the above-described methods is completely satisfactory for preparing targeted retroviral vectors. For example, the methods of U.S. patents 4,731,324 and 4,948,590 rely upon bonding an already produced compound to an existing virus or carrier. Thus, the existence of sufficient amounts of the protein and preformed virus or carrier is required.

Thus, there remains a need for viruses which contain, on their surface, proteins specific for receptors found on the membrane of targeted cells. Further, there remains a need for methods and packaging cell lines to prepare such retroviruses.

Disclosure of the Invention

Accordingly, it is an object of the present invention to provide novel viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which binds specifically to a receptor found on targeted cells.

It is another object of the present invention to provide viruses, including retroviruses, which contain on their surface at least one chimeric envelope protein which specifically binds to mammalian, including human, stem and/or progenitor cells.

It is another object of the present invention to provide viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which specifically binds to human hematopoietic stem and/or progenitor cells.

It is another object of the present invention to provide a method for preparing viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which specifically binds to a receptor found on the surface of targeted cells.

It is another object of the present invention to provide a method for preparing viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which specifically binds to mammalian, including human, stem and/or progenitor cells.

It is another object of the present invention to provide a method for preparing viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which specifically binds to human hematopoietic stem and/or progenitor cells.

It is another object of the present invention to provide human stem and/or progenitor cells which have been transduced with a genetically engineered virus, including a retrovirus, containing a therapeutic gene packaged in a virion with a

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chimeric envelope protein which specifically binds to a receptor found on the membrane of the stem and/or progenitor cell.

It is another object of the present invention to provide a method of gene therapy, in which mammalian, including human, cells are transduced with a virus, including a retrovirus, which possesses on its surface at least one chimeric envelope protein which specifically binds to the transduced cell.

It is another object of the present invention to provide a method of gene therapy, in which human stem and/or progenitor cells are transduced with a virus, including a retrovirus, which possesses on its surface at least one chimeric envelope protein which specifically binds to the human stem and/or progenitor cell.

It is another object of the present invention to provide a method of gene therapy in which human hematopoietic stem cells are transduced with a virus, including a retrovirus, possessing on its surface at least one chimeric envelope protein which specifically binds to the human hematopoietic stem and/or progenitor cells.

It is another object of the present invention to provide packaging cell lines for producing novel viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which binds specifically to a receptor found on targeted cells.

It is another object of the present invention to provide packaging cell lines for producing novel viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which binds specifically to mammalian, including human, stem and/or progenitor cells.

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It is another object of the present invention to provide packaging cell lines for producing novel viruses, including retroviruses, which possess on their surface at least one chimeric envelope protein which binds specifically to human hematopoietic stem and/or progenitor cells.

These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that viruses, including retroviruses, may be genetically engineered such that the viral membrane contains a chimeric envelope peptide which will specifically bind to a targeted cell and that such retroviruses may be used to transduce specific targeted cells.

Best Mode for Carrying Out the Invention

Thus, the present invention relates to the use of a genetically engineered retroviral packaging cell line that has altered the viral envelope such that it contains a peptide which will bind to a molecule on the membrane of the target cell. This results in the creation of stable genetically transformed mammalian, including human, cells. The present invention can be used for the transfer of any genetic information that can be engineered into a recombinant retrovirus, or any other gene transfer vector that requires a virus for delivery. The present invention also relates to a stromal cell line which supports hematopoiesis and is transfected with the retrovirus packaging vectors. Genetically modified human stem cells produced by the present invention can be applied to a wide variety of clinical diseases, as described in the preceding text. Further, the present invention also provides methodology that enhances the efficiency of genetic transfer into human progenitor cells. Additionally, the present invention includes a genetically modified human stem cell produced with the use of the present

retroviruses and/or packaging cell line.

Since tissue specificity of retrovirus infection can be conferred by virus envelope (ligand) binding to a cell receptor, and both stem and progenitor cell specific ligands and receptors are known, the construction of chimeric viral envelope/stem cell ligand molecules will confer stem and progenitor cell tropism to virus particles. For example, the HIV viral membrane gene has been cloned, and the envelope amino acids which bind to the CD4 antigen on the T-cell membrane are known (Lasky LA, et al, Cell, 50, 975-985, 1987). This knowledge provides the information needed to substitute into the gene for the HIV envelope CD4 antigen binding site a sequence that encodes the peptide that is known to bind to the cell membrane of any specific targeted cell of interest. This allows the packaging line to produce virus particles that infect target cells efficiently and selectively, because the virus particles will now encode a chimeric envelope protein which will bind specifically to the stem and/or progenitor cell receptor molecule, and will not bind to cell types which do not have the receptor.

It should be noted that by making these "designer" retroviral packaging cell lines, in vivo gene therapy may be achieved. This is especially true in view of the increased viral titers available using high density cell culture technology. As noted above, conventional packaging cell lines, both the amphotropic and GALV systems, recognize a ubiquitous cell surface protein. If the selective transduction of a target cell is the object, then any attempt to use these systems for in vivo gene therapy would be doomed to failure, because most of the infused virus would bind to irrelevant cells, and these would not be sufficient recombinant virus particles infused to infect a significant number of the target cells. Also, infection of irrelevant

1883-8, 1991), and T4 DNA ligase. The ligation reaction may be transfected into *E. coli*, and a recombinant plasmid containing the HIV envelope gene with a deletion of the bases encoding amino acids critical for CD4 binding may then be identified. There is now an Eco R1 restriction endonuclease site substituted for the deleted CD4 binding region.

A clone containing only the coding sequences of LIF may be isolated by PCR amplification of LIF (Moreau J-F et al, Nature, 336,690-692, 1988)) using the primers CCGAATTCAGCCCCCTCCCCATCACCCCTGTCAACGCCACCTGT and CCGAATTCGAAGGCCTGGGCCAACACGGCGATGATCTG. The PCR amplified LIF coding sequence is now flanked by an Eco R1 restriction endonuclease site. The PCR product may then be digested with the restriction endonuclease Eco R1 and ligated into the Eco R1 site which was introduced into the HIV ENV gene. A clone with an in-frame substitution of LIF for the CD4 binding region of the HIV ENV gene may be isolated and transfected into a cell line expressing the GAG POL genes of either an amphotropic MoMLV, or another retrovirus. This results in a packaging cell line which produces virions with a chimeric LIF/HIV envelope when transfected with a recombinant retroviral vector.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invent'on may be practiced otherwise than as specifically described herein.

Claims

1. A genetically engineered virus comprising a genetically engineered gene and having on its surface at least one chimeric envelope protein which contains a binding region specific for a cell receptor to which the naturally occurring envelope protein of the virus does not specifically bind.
2. The virus of Claim 1, which binds specifically to a single target cell receptor.
3. The virus of Claim 1, wherein said chimeric envelope protein binds specifically to mammalian stem cells, progenitor cells, or mixtures thereof.
4. The virus of Claim 3, wherein said mammalian cells are human cells.
5. The virus of Claim 1, wherein said chimeric envelope protein binds specifically to human hematopoietic stem cells, progenitor cells, or mixtures thereof.
6. A retrovirus packaging cell line, comprising packaging cells which contain the genetic information encoding for a genetically engineered virus which comprises a genetically engineered gene encoding at least one chimeric envelope protein which specifically binds to a target cell receptor to which the naturally occurring envelope protein of the virus does not bind.
7. The packaging cell line of Claim 6, wherein said chimeric envelope protein binds specifically to mammalian stem cells, progenitor cells or mixtures thereof.

8. The packaging cell line of Claim 7, wherein said mammalian cells are human cells.
9. The packaging cell line of Claim 6, wherein said chimeric envelope protein binds specifically to human hematopoietic stem cells, progenitor cells, or mixtures thereof.
10. The packaging cell line of Claim 6, wherein said packaging cells are human stromal cells.
11. In a method of gene therapy, comprising transducing mammalian cells with a retrovirus comprising the genetic information for a therapeutic gene, the improvement being that said retrovirus is packaged in a virion comprising a chimeric envelope protein which binds specifically to a cell receptor to which the naturally occurring envelope protein of the retrovirus does not bind.
12. The method of Claim 11, wherein said mammalian cells are human cells.
13. The method of Claim 12, wherein said human cells are stem cells, progenitor cells, or mixtures thereof, and said chimeric envelope protein binds specifically to human stem cells, progenitor cells, or mixtures thereof.
14. The method of Claim 12, wherein said human cells are human hematopoietic stem cells, progenitor cells, or mixtures thereof, and said chimeric envelope protein binds specifically to human hematopoietic stem cells, progenitor cells, or mixtures thereof.
15. The method of Claim 11, wherein said transducing is carried out by exposing said cells to said virus, while said

cells are being cultured by a method comprising culturing said cells in a liquid culture medium which is replaced at a rate of about 1 ml of medium per 1 ml of culture per about 24 to about 48 hours, and removing metabolic products and replenishing depleted nutrients, while maintaining said culture under physiologically acceptable conditions.

16. The method of Claim 15, wherein said cells are human cells.

17. The method of Claim 11, wherein said transducing is carried out *in vivo*.

18. Human stem and/or progenitor cells which have been transduced with a genetically engineered virus comprising a genetically engineered gene packaged in virion with a chimeric envelope protein which contains a binding region specific for a cell receptor to which the naturally occurring envelope protein of the virus does not specifically bind.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00072

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : C12N 5/00, 15/00; A61K 37/00, 31/70

US CL : 435/240.1, 320.1; 514/44; 424/93B

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.1, 320.1; 514/44; 424/93B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cell, Volume 63, issued 05 October 1990, F.H. Martin et al, "Primary Structure and Functional Expression of Rat and Human Stem Cell Factor cDNAs", pages 203-211, see entire document.	1-18
X	Journal of Virology, Volume 63, No. 5, issued May 1989, "Formation of Infectious Hybrid Virions with Gibbon Ape Leukemia Virus and Human T-Cell Leukemia Virus Retroviral Envelope Glycoproteins and the gag and pol Proteins of Moloney Murine Leukemia Virus", pages 2374-2378, see entire document.	1-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00072

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings National Academy of Sciences, Volume 87, issued January 1990, J. M. Wilson et al, "Expression of Human Adenosine Deaminase in Mice Reconstituted with Retrovirus-Transduced Hematopoietic Stem Cells", pages 439-443, see entire document.	1-18
X	Blood, Volume 69, No. 2, issued February 1987, Hogge et al, "Gene Transfer to Primary Normal and Malignant Human Hemopoietic Progenitors Using Recombinant Retroviruses", pages 611-617, see entire document.	1-18